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Evaluating the effects of metal oxide nanoparticles (TiO_2, Al_2O_3, SiO_2 and CeO_2) on anammox process: Performance, microflora and sludge properties

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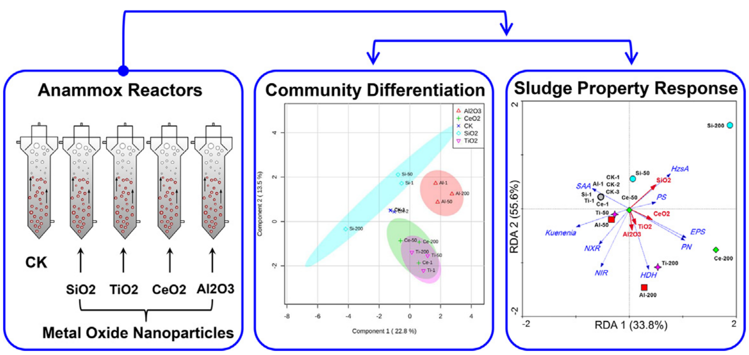
GRAPHICAL ABSTRACT



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ABSTRACT

The increasing use of engineered metal oxide nanoparticles (MONPs) in consumer products raises great concerns about their environmental impacts, but their potential impacts on anaerobic ammonium oxidation (anammox) process in wastewater treatment remain unclear. In this study, the presence of MONPs (1, 50, 200 $mg\,L^{-1}$) exhibited no visible effects on the nitrogen removal performance of anammox reactors, but high levels (200 $mg\,L^{-1}$) of SiO_2NPs, Al_2O_3NPs and CeO_2NPs had a distinct effect on shaping the anammox community. Long-term exposure of MONPs caused different responses in the relative abundance of *Ca. Kuenenia*, the level of functional gene *Hzs*A and the activities of three key enzymes involved in anammox metabolism, but no significant inhibition effects on specific anammox activity were detected. Overall, the effects of MONPs on anammox community structure and sludge properties depended on their types and levels and followed the order $SiO_2 > CeO_2 > Al_2O_3 > TiO_2$.

1. Introduction

Given the rapid commercialization and broad application of nanotechnology, metal oxide nanoparticles (MONPs), such as titanium dioxide (TiO_2), ceria dioxide (CeO_2), aluminum oxide (Al_2O_3) and silicon dioxide (SiO_2), have been widely applied in commercial and industrial products owing to their unique properties (Li et al., 2017a; Mu et al., 2011). The use of TiO_2NPs is widespread in catalysts, cosmetics, paints, sunscreens and plastics due to optical, catalytic and antibacterial properties (Mu et al., 2011). Al_2O_3NPs are widely applied in catalysts,

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sunscreens, and additives to paints, rubbers, and plastics. CeO_2NPs have been widely used as abrasives, ultraviolet absorbents and fuel additives (Ma et al., 2013; You et al., 2017). Although silicon is a metalloid, nanoscale SiO_2 is generally discussed as a type of metal oxide because of its stability and similar chemical properties to other oxides, such as TiO_2 and Al_2O_3 (Walden and Zhang, 2016). Given the properties of reinforcement, thickening, thixotropic, insulation and extinction, SiO_2NPs are valuable components of rubbers, plastics, coatings, adhesives, sealants and other polymer industries (Li et al., 2017a).

However, MONPs are inevitably released into the environment at different stages of their life cycle, including production, manufacturing, and consumption. For example, MONPs are released from the washing of textiles, weathering of paints, and other consumer products, such as sunscreens and cosmetics (Mohanty et al., 2014). Ultimately, MONPs released from different nanomaterials are finding their way to sewage pipes and subsequently wastewater treatment plants (WWTPs) (Wang et al., 2017). Among several treatment processes within WWTPs, including sedimentation, biological treatment, and sludge processing, activated sludge can remove most of MONPs in wastewater efficiently (Walden and Zhang, 2016). Given the potential toxicity of MONPs, their impacts on functional microorganisms in biological treatment systems have drawn increasing concerns from the public (Wang and Chen, 2016; Wang et al., 2017).

Indeed, numerous studies have recently revealed the adverse effects of MONPs on wastewater biological treatment. For example, long-term exposure to 10–60 $mg\,L^{-1}$ TiO_2NPs obviously impacted the treatment performance of organic matter, nitrogen and phosphorus as well as the microbial community of activated sludge (Li et al., 2017b). In addition, 5 $mg\,L^{-1}$ SiO_2NP significantly reduced the oxygen uptake rate of activated sludge and altered the composition of microbial membrane lipids (Sibag et al., 2015). Additionally, 5 $mg\,L^{-1}$ CeO_2NP significantly affected the specific filtration resistance during sludge dewatering process, which might increase disposal costs (You et al., 2017). Approximately 50–500 $mg\,L^{-1}$ CeO_2NP caused strong inhibition on methanogenesis and partial nitrification processes (García et al., 2012). Ma et al. (2013) also stated that in the presence of CeO_2NPs at 150 $mg\,g^{-1}$VSS, the acidification process was inhibited by 35% for anaerobic granular sludge using glucose as a carbon source. However, there are other studies that hold divergent opinions. Ma et al. (2015) found that long-term exposure to TiO_2NPs or CeO_2NPs at 0.1–20 $mg\,L^{-1}$ did not inhibit the nitrification function of sequencing batch reactors (Ma et al., 2015). Another study noted that the presence of TiO_2NPs, Al_2O_3NPs or SiO_2NPs up to 150 $mg\,g^{-1}$SS exhibited no inhibitory effects on the anaerobic digestion process of waste activated sludge (Mu et al., 2011). García et al. (2012) reported no significant inhibition effects on methanogenesis and partial nitrification processes upon exposure to 200–1010 $mg\,L^{-1}$ TiO_2NPs. Similar results were also observed by Ma et al. (2013), wherein the exposure to150 $mg\,g^{-1}$VSS CeO_2NPs did not inhibit the methanogenesis process. Overall, the impact of MONPs on biological treatment depended on the species of the functional microorganism and the experimental conditions. However, limited information is available on the potential effects of MONPs on anaerobic ammonium oxidation (anammox) process (Zhang et al., 2018a, 2017a).

Anammox process, wherein autotrophic anammox bacteria directly convert ammonium to N_2 using nitrite as the electron acceptor under anaerobic conditions, is one of the most advanced technologies in biological nitrogen removal (BNR) processes. Given their advantage of energy efficiency, anammox-based processes are widely accepted as novel alternatives to traditional BNR processes (Kartal et al., 2010; van Loosdrecht and Brdjanovic, 2014), and these processes have made great progress in the full-scale application of sidestream treatment according to the recent application survey (Lackner et al., 2014). The feasibility of anammox-based processes for mainstream treatment is gradually confirmed by recent studies (Laureni et al., 2016; Lotti et al., 2014; Reino et al., 2018). In China, anammox has been selected as an indispensable part in both sidestream and mainstream in the 'new concept sewage treatment plants' of the future.

Therefore, this study aims to investigate the individual effects of four MONPs (TiO_2, Al_2O_3, SiO_2 and CeO_2) on anammox process and understand the mechanisms. First, the nitrogen removal performance of anammox reactors exposed to MONPs at various levels was evaluated. In addition, the response of anammox consortia was tracked by high-throughput sequencing. Furthermore, the evolution of sludge properties, mainly including specific anammox activity (SAA), functional gene abundance, enzyme activities and extracellular polymeric substances (EPS), was also tracked. Finally, the effect mechanisms were explained with the aid of redundancy analysis (RDA) with Pearson's correlation analysis.

2. Materials and methods

2.1. Origin of anammox biomass and nanoparticles

The mature anammox granules used for continuous-flow experiments were collected from high-loaded up-flow anaerobic sludge blanket (UASB) reactors. These parent reactors have been operated stably under thermostatic (35 ± 1 °C) conditions for more than two years. The seeding sludge with a mean diameter of 2.5 ± 1.4 mm possessed an SAA of 685.3 ± 45.6 $mgTN\,g^{-1}$ volatile suspended solids (VSS) d^{-1} and an EPS content of 206.3 ± 14.6 $mg\,g^{-1}$VSS.

Commercially produced SiO_2NPs (99.5% purity, 30 ± 5 nm), anatase TiO_2NPs (99.8% purity, 60 nm, hydrophilic), CeO_2NPs (99.5% purity, 20–50 nm), and α-Al_2O_3NPs (99.9% purity, 30 nm, hydrophilic) were obtained from Aladdin Reagent Co. Ltd., China. The stock suspensions of MONPs (2 $g\,L^{-1}$) were prepared according to previous studies (Mu et al., 2011; Zhang et al., 2017a). In brief, the nano powder was suspended in distilled water (pH 7.5), and 0.1 mM sodium dodecyl benzene sulfonate was added as a dispersing reagent to strengthen its stability. The stock suspensions were homogenized in an ultrasonic bath (25 °C, 250 W, 40 kHz) before addition to the influent of reactors for subsequent experiments.

2.2. Experimental setup

The continuous-flow experiments were conducted with five laboratory-scale UASB reactors. Each reactor (fabricated from Plexiglas) had a working volume of 1.0 L with the same inner diameter (6 cm). Briefly, 1.0 L of anammox granules was inoculated to each reactor to obtain an initial biomass concentration of approximately 20 $gVSS\,L^{-1}$. These reactors were then operated in the dark and thermostatic (35 ± 1 °C) room. The synthetic wastewater containing substrates (560 $mgTN\,L^{-1}$), minerals and trace elements was pumped into the reactors, in which equimolar NH_4^+ and NO_2^- were supplied as substrates (details are shown in the Supplementary materials) (Zhang et al., 2018b). The pH value of influent was controlled at approximately 7.8, and the hydraulic retention time was fixed at 1.2 h throughout the experimental phases. The levels of dissolved oxygen in five reactors were less than the detection limit (0.1 $mg\,L^{-1}$). Although the current concentration of MONPs in municipal wastewater was estimated in a range of $\mu g\,L^{-1}$ to $mg\,L^{-1}$, MONPs may accumulate in activated sludge owing to their high affinity for sludge and then release to sludge digestion liquid (Musee et al., 2011; Walden and Zhang, 2016). Therefore, besides the effects of MONPs at environmentally relevant levels (1 $mg\,L^{-1}$) on anammox process, the potential effects of higher levels of MONPs (50 and 200 $mg\,L^{-1}$) were also addressed to make a comprehensive judgment per previous suggestions (Mu et al., 2011, 2012). Each concentration of MONPs lasted for 30 days, and the specific sludge loads of MONPs were estimated as 0.05, 2.5 and 10 $mg\,g^{-1}$VSS. Sludge exceeding the working volume was discharged from the reactors periodically to maintain a relatively stable biomass concentration.